rhBMP-2 significantly enhances guided bone regeneration

Ulf M. E. Wikesjö
Mohammed Qahash
Robert C. Thomson
Alonzo D. Cook
Michael D. Rohrer
John M. Wozney
W. Ross Hardwick

Authors’ affiliations:
Ulf M. E. Wikesjö, Mohammed Qahash, Laboratory for Applied Periodontal and Craniofacial Regeneration, Department of Periodontology, Temple University School of Dentistry, Philadelphia, PA, USA
Michael D. Rohrer, Division of Oral and Maxillofacial Pathology, University of Minnesota School of Dentistry, Minneapolis, MN, USA
John M. Wozney, Musculoskeletal Sciences, Bone Biology & Applications, Wyeth Research, Cambridge, MA, USA

Correspondence to:
Dr Ulf M. E. Wikesjö
Laboratory for Applied Periodontal and Craniofacial Regeneration
Temple University School of Dentistry
Department of Periodontology
3223 North Broad Street
Philadelphia, PA 19140, USA
Tel.: +1 215 707-5320
e-mail: ulf.wikesjo@temple.edu
web site: http://www.temple.edu/dentistry/perio/index.htm

Key words: tissue engineering, differentiation factors, metal implants, seroma

Abstract

Background: Previous studies have shown a limited potential for bone augmentation following guided bone regeneration (GBR) in horizontal alveolar defects. Surgical implantation of recombinant human bone morphogenetic protein-2 (rhBMP-2) in an absorbable collagen sponge carrier (ACS) significantly enhances bone regeneration in such defects; however, sufficient quantities of bone for implant dentistry are not routinely obtained. The objective of this study was to evaluate the potential of rhBMP-2/ACS to enhance GBR using a space-providing, macro-porous expanded polytetrafluoroethylene (ePTFE) device.

Methods: Bilateral, critical size, supra-alveolar, peri-implant defects were surgically created in four Hound Labrador mongrel dogs. Two turned and one surface-etched 10-mm titanium dental implant were placed 5 mm into the surgically reduced alveolar ridge creating 5-mm supra-alveolar defects. rhBMP-2/ACS (rhBMP-2 at 0.2 mg/ml) or buffer/ACS was randomly assigned to left and right jaw quadrants in subsequent animals. The space-providing, macro-porous ePTFE device was placed to cover rhBMP-2/ACS and control constructs and dental implants. Gingival flaps were advanced for primary wound closure. The animals were euthanized at 8 weeks postsurgery for histologic and histometric analysis.

Results: Bone formation was significantly enhanced in defects receiving rhBMP-2/ACS compared to control. Vertical bone gain averaged (± SD) 4.7 ± 0.3 and 4.8 ± 0.1 mm, and new bone area 10.3 ± 2.0 and 8.0 ± 2.5 mm² at turned and surface-etched dental implants, respectively. Corresponding values for the control were 1.8 ± 2.0 and 1.3 ± 1.3 mm, and 1.8 ± 1.3 and 1.2 ± 0.6 mm². Bone–implant contact in rhBMP-2-induced bone averaged 6.4 ± 1.4% and 9.6 ± 7.5% for turned and surface-etched dental implants, respectively (P = 0.399). Corresponding values for the control were 14.6 ± 19.4% and 23.7 ± 9.7% (P = 0.473). Bone–implant contact in resident bone ranged between 43% and 58% without significant differences between dental implant surfaces.

Conclusions: rhBMP-2/ACS significantly enhances GBR at turned and surface-etched dental implants. The dental implant surface technology does not appear to substantially influence bone formation.

Biologic studies have evaluated guided bone regeneration (GBR) for augmentation of the alveolar process. Schenk et al. [1994] demonstrated a significant potential for regeneration of alveolar bone using a saddle-type intrabony defect and an occlusive GBR device. However, bone regeneration was not complete following a 16-week healing interval. Buser et al. [1995] placed dental implants into the regenerated alveolar bone and observed dental implant osseo-integration. In contrast, Caplanis et al. [1997] evaluating GBR using supra-alveolar peri-implant defects observed limited vertical
augmentation of the alveolar process following a 16-week healing interval. The majority of the space underneath the GBR device was filled with dense connective tissue. The addition of an allogeneic demineralized freeze-dried bone (DFDBA) biomaterial did not increase bone formation. The biomaterial remained encapsulated in fibrous connective tissue underneath the device. Apparently, outcomes following GBR are considerably dependent on the defect morphology. Defects with smaller dimensions and intrabony defects have a higher regenerative potential than larger and predominantly supra-alveolar defects. Also, regeneration following GBR may require considerable time. In addition, biomaterials such as DFDBA may not have a decisive effect on alveolar bone formation.


Sigurdsson et al. (1997) evaluated the potential of rhBMP-2 in an absorbable collagen sponge [ACS] carrier for vertical alveolar augmentation and immediate dental implant osseointegration using the supra-alveolar defect model introduced by Caplanis et al. (1997). Briefly, three 10-mm titanium fixtures were placed 5 mm into the surgically reduced edentulated alveolar crest leaving 5 mm of the dental implant in a supra-alveolar position. Alternate jaw quadrants in consecutive animals were implanted with rhBMP-2/ACS or ACS alone (control). Fixtures and rhBMP-2 or control constructs were submerged under the mucogingival flaps. The healing interval was 16 weeks. Surgical implantation of rhBMP-2/ACS resulted in vertical ridge augmentation encompassing the entire height of the 5-mm, peri-implant defect. Vertical alveolar bone augmentation in the control averaged 0.5 mm! However, the rhBMP-2/ACS-induced alveolar ridge varied considerably in volume and geometry within and between animals. Bone regeneration area averaged 6.1 ± 6.3 and 0.2 ± 0.2 mm² for rhBMP-2/ACS and control sites, respectively. It was suggested that the ACS carrier exhibits limited mechanical resistance to compressive forces from or transmitted through the mucogingival flaps resulting in lack of space maintenance for bone formation in particular for challenging onlay settings such as in the supraalveolar peri-implant defect model. Barboza et al. (2000) and Tatakis et al. (2002) have subsequently made similar observations regarding rhBMP-2/ACS.

The overall objective of this research was to evaluate the effect of a space-providing, macro-porous GBR device on rhBMP-2/ACS-induced alveolar augmentation and immediate dental implant osseointegration. The objective of this study was to evaluate the potential of rhBMP-2/ACS to enhance GBR using a space-providing, macro-porous expanded polytetrafluoroethylene (ePTFE) device.

Material and methods

Animals

Four male Hound Labrador mongrel dogs, aged 10–12 months, weight approximately 20 kg, obtained from USDA-approved dealer, were used. Animal selection and management, surgery protocol, and alveolar defect preparation followed routines approved by the Animal Care and Use Committee, W.L. Gore & Associates, Inc. Flagstaff, AZ, USA. The animals had access to standard laboratory diet and water until the beginning of the study. Oral prophylaxis was performed within 2 weeks prior to experimental surgeries.

Macro-porous space-providing devices

A space-providing, macro-porous ePTFE barrier device [Reinforced GORE-TEX® ePTFE, W.L. Gore & Associates Inc., Flagstaff, AZ, USA] was used. The macro-porous device, reinforced with a laminated polypropylene mesh, was custom-made for the critical size, supra-alveolar, peri-implant defect model and has laser-etched 300-µm pores at 0.8-mm intervals (center to center) allowing for penetration of the gingival connective tissue. These device characteristics have been shown to support alveolar bone and cementum regeneration in the supra-alveolar periodontal defect model [Wikesjö et al. 2003c].

rhBMP-2/ACS

Using aseptic routines, 8.0 mg of lyophilized rhBMP-2 [Wyeth Research, Cambridge, MA, USA] was reconstituted with 1.8 ml sterile water [Baxter, McGaw Park, IL, USA] and diluted with 0.2 ml MFR 00842 buffer (5 mM L-glutamic acid, 2.5% glycerine, 0.5% sucrose, 0.01% polysorbate 80, pH 4.5) [Wyeth Research, Cambridge, MA, USA] to create a 4.0 mg/ml solution. On the day of surgery, for each animal, 0.1 ml was withdrawn from the 4.0 mg/ml rhBMP-2 solution and added to 1.9 ml MFR 00842 buffer to create an aliquot of 2.0 ml at 0.2 mg/ml.

A sterile 1 × 2 inch absorbable collagen sponge [ACS; Helistat™, Integra Life Sciences, Plainsboro, NJ, USA] was removed from the outer tray and sectioned. Five sections measuring 32 × 10 × 3.4 mm [dry dimensions] were cut from the 1 × 2 inch ACS. A central longitudinal slit, 28 mm long, was cut in four of the sections and the fifth section was divided into smaller segments. The 2.0 ml aliquot of the 0.2 mg/ml rhBMP-2 solution (0.4 mg rhBMP-2) was withdrawn and uniformly dispensed over the entire surface of the cut ACS sections. Following a 30-min binding period, the rhBMP-2 soaked ACS sections were placed into the supra-alveolar peri-implant defect. The control construct was created in exactly the same manner except that 2.0 ml of MFR 00842 buffer was used to soak-load the ACS.

Dental implants

Turned or surface acid-etched, commercially pure titanium dental implants
[Implants Innovations, Inc., Palm Beach Gardens, FL, USA] were used, the implant surface technologies being representative of the supplier’s clinical products. The custom dental implants were manufactured with a reference thread 5 mm from the top surface. The reference thread was designed to facilitate the surgical placement of the implants, leaving 5 mm of the dental implant in a supra-alveolar position, and to serve as a reference point for the histologic and histometric analysis.

Surgical protocol

Food was withheld the night before surgical procedures. The animals were pre-medicated with atropine [0.02 mg/kg i.m.], buprenorphine [0.04 mg/kg i.m.], and flunixin meglumine [0.1 mg/kg i.v.]. A prophylactic antibiotic [cefazolin; 22.0 mg/kg i.v.] was administered. General anesthesia was induced with diazepam [0.2 mg/kg i.v.] and ketamine [6.0 mg/kg i.v.]. An endotracheal tube was placed and the animals were maintained on isoflurane gas [1–2 %] in 100% oxygen using positive pressure ventilation. A sterile catheter was placed and the animals received a slow constant rate infusion of lactated Ringer’s solution [10–20 ml/kg/h i.v.] to maintain hydration while anesthetized. Routine dental infiltration anesthesia was used at the surgical sites.

Discriminating, critical size, supra-alveolar peri-implant defects were created in the mandibular premolar region in the left and right jaw quadrants [Fig. 1 | Wikesjö et al. 2001]. Briefly, buccal and lingual mucoperiosteal flaps were reflected following buccal and lingual sulcular incisions from the canine tooth to the second molar. Alveolar bone was removed around the circumferenece of the first, second, third, and fourth premolar teeth using chisels and water-cooled rotating burs to a level of 6 mm from the CEJ and the teeth were then extracted. The first molar was amputated at the level of the surgically reduced alveolar crest. The custom titanium dental implants, two turned and one surface acid-etched, were placed 5 mm within the surgically reduced alveolar ridge to the level of the reference thread, creating 5-mm, supra-alveolar, peri-implant defects. The two turned dental implants were placed in the region of the fourth premolar tooth and the surface-acid etched dental implant was placed in the region of the third premolar tooth in each side of the mandible. The maxillary first, second, and third premolar teeth were surgically extracted, and the maxillary fourth premolars were reduced in height and exposed pulpal tissues sealed [Cavit®, ESPE, Seefeld/Oberbayern, Germany]; this is to avoid potential trauma from the maxillary teeth to the experimental mandibular sites postsurgery.

Experimental protocol

Contralateral peri-implant defects in subsequent animals were implanted with rhBMP-2/ACS or buffer/ACS. The constructs were placed over the titanium fixtures in layers for a total volume of 2.0 ml/defect. The space-providing, macroporous ePTFE device was placed to cover the dental implants and rhBMP-2/ACS or buffer/ACS constructs. The device was fixed to the alveolar bone with medical-grade stainless-steel tacks [FRIOS® Augmentation system, Friadent, Mannheim, Germany].

Following device placement, the periosteum was fenestrated at the base of the flaps to allow primary wound closure with tension-free flap apposition. The flaps were advanced, the flap margins being adapted 3–4 mm coronal to the macro-porous ePTFE device and sutured (GORE-TEX™ Suture CV5, W.L. Gore & Associates Inc., Flagstaff, AZ, USA). Intra-surgery photographs were obtained prior to and immediately after placement of the ePTFE device, and following wound closure.

Post-surgery protocol

Animals were fed a canned soft dog-food diet the first 14 days postsurgery. Thereafter, the animals received standard laboratory diet soaked in warm water until thoroughly soft. The animals received buprenorphine [0.04 mg/kg i.v., i.m., or s.q] every 5 h for analgesia for the first few days postsurgery. A broad-spectrum antibiotic [enrofloxacin; 2.5 mg/kg, i.m., b.i.d.] was used for infection control for 14 days. Plaque control was maintained by twice daily topical application of chlorhexidine [chlorhexidine gluconate 20% (Xtrium Laboratories, Inc., Chicago, IL, USA), 40 ml of a 2% solution] until gingival suture removal, thereafter, once daily [Monday–Friday] until completion of the study.

Radiographs were obtained immediately postsurgery, and at 4 and 8 weeks postsurgery. Gingival sutures were removed under sedation at approximately 10 days postsurgery. Observations of experimental sites with regard to gingival health, maintenance of suture line closure, edema, and evidence of tissue necrosis or infection were

Fig. 1. Bilateral, critical size, supra-alveolar peri-implant defects were surgically created immediately post-extraction in the posterior mandible. Two turned and one surface-etched 10-mm titanium dental implants were placed 5 mm into the surgically reduced alveolar ridge creating 5-mm supra-alveolar peri-implant defects (a). One defect site in each animal received rhBMP-2 [0.2 mg/ml]/ACS and the contralateral site buffer/ACS (b). A space-providing, macro-porous ePTFE device was placed to cover the rhBMP-2/ACS and control construct and dental implants (c). Gingival flaps were advanced for primary wound closure (d). Healing at 8 weeks appeared normal. In sites receiving buffer/ACS (e), the ridge extension appeared slightly smaller than that observed in sites receiving rhBMP-2/ACS (f).
made daily until suture removal, and at least twice weekly thereafter and recorded.

The animals were anesthetized and euthanized at 8 weeks postsurgery by an intravenous injection of concentrated sodium pentobarbital. Following euthanasia, dental implants with surrounding soft and hard tissues were removed en bloc. ePTFE devices were not removed during the healing interval.

**Histological processing**

Tissue blocks including titanium fixtures, bone, and soft tissue were removed. The tissue blocks were fixed in 10% buffered formalin for 3–5 days, dehydrated in alcohol, and embedded in methylmethacrylate resin (Technovit 7200 VLC, Kulzer, Germany). The implants were cut midaxially in a buccal–lingual plane into sections of 200 μm thickness using the cutting–grinding technique [EXAKT Apparatebau, Norderstedt, Germany], and subsequently ground and polished to a final thickness of approximately 40 μm (Donath & Breuner 1982; Rohrer & Schubert 1992). The sections were stained with Stevenel’s blue and van Gieson’s picro fuchsin. The most central section for each implant was used for the histologic and histometric analysis.

**Analysis**

Using a magnifier/masking device (Viewscope 2 x, Flow X-Ray Corp., Hempstead, NY, USA), dental radiographs from immediately postsurgery and from 4 and 8 weeks postsurgery were evaluated by two examiners for alveolar augmentation, cortical bone formation and resorption, seroma formation, peri-implant bone formation, and bone formation relative to the macro-porous ePTFE device.

The histopathologic evaluation by the two examiners included observations of new bone formation and resorption, woven and lamellar bone, cortex formation, seroma formation, fibrovascular tissue and marrow, inflammatory responses, residual ACS, vascularity, peri-implant bone formation, and bone formation relative to the macro-porous ePTFE device.

The most central section from each dental implant was used for the histometric analysis. Analysis was performed using incandescent and polarized light microscopy [BX 60, Olympus America, Inc., Melville, NY, USA], a microscope digital camera system [DP10, Olympus America, Inc., Melville, NY, USA], and a PC-based image analysis system [Image-Pro Plus™, Media Cybernetic, Silver Springs, MD, USA] by one experienced investigator masked to the specific experimental conditions. The following measurements were recorded for the buccal and lingual surfaces of each dental implant:

- **Defect height**: distance between the reference thread and the dental implant platform.
- **Device (height)**: distance between the reference thread and the point where the device contacted the dental implant or the dental implant platform.
- **Wound area**: area circumscribed by the implant, the device, and the base of the defect at the level of the reference thread.
- **Bone regeneration (height)**: distance between the reference thread and the vertical extension of newly formed bone along the implant.
- **Bone regeneration (area)**: area represented by new alveolar bone along the implant.
- **Bone density (new bone)**: ratio of new bone/marrow spaces.
- **Bone density (resident bone)**: ratio of bone/marrow spaces adjacent to the dental implant.
- **Osseointegration new bone**: percent bone–implant contact (BIC) as measured between the reference thread and the point of the coronal extension of newly formed bone along the implant.
- **Osseointegration resident bone**: percent BIC within the alveolar base as measured from the apical aspect of the reference thread to the apex of the dental implant.

**Statistical analysis**

Summary statistics (means ± SD) based on animal means for the experimental conditions were calculated using the selected sections. Paired t-tests were performed to evaluate differences between treatment conditions (N = 4). Significance was accepted at a probability level of P ≤ 0.05.

**Results**

**Clinical observations**

At suture removal, approximately 10 days postsurgery, jaw quadrants receiving rhBMP-2/ACS exhibited redness and moderate swelling. At 4 weeks, swelling and redness were significantly reduced in these jaw quadrants. However, bluish vesicles characteristic of seroma formation were observed in one animal. At 8 weeks, healing appeared within normal limits in all jaw quadrants receiving rhBMP-2/ACS. None of the sites exhibited wound failure or defect exposure (Fig. 1).

Jaw quadrants receiving the space-providing, macro-porous ePTFE device without rhBMP-2/ACS showed healing within normal limits with considerably less swelling and redness compared to contralateral sites receiving rhBMP-2/ACS at suture removal. At 4 and 8 weeks, healing in the controls appeared within normal limits. The ridge dimensions appeared smaller than that observed in sites receiving rhBMP-2/ACS. None of the sites exhibited wound failure or defect exposure.

**Radiographic observations**

Jaw quadrants receiving rhBMP-2/ACS exhibited a suggestion of bone formation approximating the outline of the space-providing, macro-porous ePTFE device and extending 2–3 mm above the top surface of the dental implants at 4 weeks postsurgery. Radiolucencies adjacent to the dental implant surfaces were apparent within the newly formed bone in three animals (Fig. 2). The radiolucencies varied in size and location to, in the extreme case, encompass almost the entire space provided by the macro-porous ePTFE device. The resident bone at the base of the defect exhibited evidence of resorption not extending beyond the first thread apical to the reference thread. At the 8-week observation, increased radiopacity within the outline of the ePTFE devices suggested formation of fine trabecular bone. The radiolucencies observed at week 4 appeared, in part, resolved by bone formation.

Jaw quadrants receiving the macro-porous ePTFE device without rhBMP-2/ACS showed no bone formation at 4 weeks postsurgery except in one animal. At 8 weeks, these sites exhibited suggestion of...
There was only limited inflammatory reaction associated with the macro-porous ePTFE device. Blood vessels were observed passing through the pores (Fig. 4). There was no evidence of residual ACS. Jaw quadrants receiving buffer/ACS exhibited bone formation limited to the first few threads above the reference thread. In contrast, defect sites receiving rhBMP-2/ACS showed radiopacity surrounding the entire implants exceeding the level of the implant platform. Note the radiolucency approximating the most coronal part of the central implant suggesting seroma formation [b].

Fig. 2. Representative radiographs at the 8-week healing interval of contralateral jaw quadrants receiving the space-providing, macro-porous ePTFE device with buffer/ACS [a] or rhBMP-2/ACS [b]. Defect sites receiving buffer/ACS exhibited bone formation limited to the first few threads above the reference thread. In contrast, defect sites receiving rhBMP-2/ACS showed radiopacity surrounding the entire implants exceeding the level of the implant platform. Note the radiolucency approximating the most coronal part of the central implant suggesting seroma formation [b].

Histologic observations

Jaw quadrants receiving rhBMP-2/ACS exhibited sparsely trabecular bone formation completely filling the space underneath the macro-porous ePTFE device approximating the dental implant, however, with limited BIC [Figs 3–5]. The larger volume underneath the device was filled with fibrovascular marrow and included apparent osteogenic activity [Fig. 3]. Two animals exhibited seroma formation, which appeared more prominent around the posteriorly placed dental implants. There was only limited inflammatory reaction associated with the macro-porous ePTFE device. Blood vessels were observed corresponding to 96% and 98% of the defect height at turned and surface-etched dental implants, respectively. The corresponding values for the control were 1.8 ± 2.0 and 1.3 ± 1.3 mm and 37% and 26%. New bone area averaged 10.3 ± 2.0 and 8.0 ± 2.5 mm² at turned and surface-etched dental implants, respectively, at defect sites receiving rhBMP-2/ACS (Table 2). The corresponding values for the control were significantly smaller averaging 1.8 ± 1.3 and 1.2 ± 0.6 mm² [P ≤ 0.01].

Comparisons between dental implant osseointegration at turned and etched surface technologies are shown in Tables 3 and 4. BIC at sites receiving rhBMP-2/ACS averaged 6.4 ± 1.4% and 9.6 ± 7.5% for turned and surface-etched dental implants, respectively [P = 0.399] (Table 3). Corresponding values for control sites were 14.6 ± 19.4% and 23.7 ± 9.7% [P = 0.473]. BIC in resident bone ranged between 43% and 58%, without significant differences between dental implant surface technologies (Table 4).

Discussion

The objective of this study was to evaluate the potential of rhBMP-2/ACS to enhance GBR. Supra-alveolar, peri-implant defects were created in four Hound Labrador dogs. Two turned and one surface-etched 10-mm titanium dental implants were placed 5 mm into the surgically reduced alveolar ridge creating 5-mm supra-alveolar, peri-implant defects. rhBMP-2/ACS and the control, buffer/ACS, were randomly assigned to left and right jaw quadrants in subsequent animals. A space-providing, macro-porous ePTFE device was placed over the rhBMP-2/ACS and control constructs and dental implants. The mucogingival flaps were advanced for primary wound closure. The animals were euthanized at 8 weeks postsurgery for histologic and histometric analysis. Bone formation was significantly enhanced in defects receiving rhBMP-2/ACS compared to control. BIC ranged between 6% and 10% for turned and surface-etched dental implants in rhBMP-2/ACS-induced bone and 15% and 24% in controls. BIC in resident bone ranged between 43% and 58%, without significant differences between dental implant surface technologies. The results
suggest that rhBMP-2/ACS significantly enhances bone formation under conditions for GBR.

Canine and non-human primate preclinical models have been used to evaluate alveolar ridge augmentation protocols and dental implant osseointegration. These models range from extraction sites and intrabony defects to discriminating critical size supra-alveolar peri-implant defects. Extraction sites and intrabony defects offer significantly greater osteogenic resources than a supra-alveolar defect, and thus have a relatively large potential for healing without additional therapy and commonly exhibit complete fill following GBR. However, GBR, as a stand-alone therapy, has a limited potential for vertical augmentation of the alveolar ridge (Simion et al. 1994; Jovanovic et al. 1995; Caplanis et al. 1997; Schliephake et al. 1999). Caplanis et al. (1997) using a space-providing, occlusive ePTFE device showed that vertical bone augmentation only averaged 1.1 mm of 5-mm supra-alveolar ridge defects following a 16-week healing interval. The addition of DFDBA did not improve bone augmentation. This preclinical model appears to be a discriminating model for evaluation of GBR technologies, bone grafts, bone derivatives, and substitutes, and evaluation of biologic factors for alveolar augmentation. In addition, the model appears to be useful for evaluating BIC between immediate dental implants and the newly formed bone as well as the contiguous resident bone. The model also allows comparisons between dental implant technologies. In this study, we improved the precision of the supra-alveolar peri-implant defect model by using custom-made dental implants with a reference thread at the 5-mm mid-point. The reference thread served two purposes. It facilitated accurate placement of the dental implant and precise assessment of the bone healing in the histometric analysis.

Limited peri-implant bone augmentation has been reported following reconstructive surgery without GBR devices in large supra-alveolar peri-implant defects (Sigurdsson et al. 1997). Vertical ridge augmentation amounted to 0.5 mm and new alveolar bone area averaged 0.2 mm². Application of an occlusive GBR device to similar peri-implant defects with or without DFDBA also resulted in minimal improvements in vertical peri-implant bone augmentation (Caplanis et al. 1997). In the present study, bone formation [height and area] at a majority of sites receiving the macro-porous ePTFE device and buffer/ACS was limited and similar to that observed in previous studies (Caplanis et al. 1997; Sigurdsson et al. 1997). It is important to note that the application of the macro-porous ePTFE device resulted in as much vertical bone augmentation as that observed following use of an occlusive ePTFE device (Caplanis et al. 1997). These observations corroborate those from a recent study evaluating the regenerative potential of occlusive and macro-porous ePTFE devices in a periodontal defect model, suggesting that device occlusivity does not appear to be a critical prerequisite for guided tissue regeneration (GTR) (Wikesjö et al. 2003e). Interestingly, bone
formation in supra-alveolar periodontal defects appears significantly greater than in the corresponding supra-alveolar peri-implant defects [Polimeni et al. 2002]. In contrast, when rhBMP-2/ACS was applied to the supra-alveolar peri-implant defects in the present study, the newly formed bone completely surrounded the dental implants to approximate the contour of the macro-porous ePTFE device. Bone formation rarely progressed beyond the outline of the device. Thus, the result of the present study suggests that rhBMP-2/ACS will significantly enhance the regenerative potential of GBR for vertical ridge augmentation procedures; however, extended healing intervals are necessary to evaluate how the newly induced bone meets functional demands.

There were significant differences in bone density and BIC between rhBMP-2/ACS-induced bone and the newly formed bone in the control sites or the resident bone at the base of the defect. This observation corroborates observations by Sigurdsson et al. (1997) and Tatakis et al. (2002) evaluating rhBMP-2/ACS in the same model system, however, without GBR devices. Apparently, the macro-porous ePTFE device did not have a significant influence on rhBMP-2/ACS-induced bone density and consequently BIC following the 8-week healing interval. Although there were no statistically significant differences in BIC between dental implant surfaces, BIC tended to be higher among surface-etched implants even in the rhBMP-2/ACS-induced low-density bone compared to that at turned dental implants.

A seroma is defined as a sterile accumulation of serum in a circumscribed location in tissue. The difference between a seroma and an abscess is that an abscess involves the presence of white blood cells, bacteria, and breakdown products of both. A seroma, on the other hand, is serum that has accumulated in a dead space in the tissue. It has been suggested to be the result of tissue insult, the product of tissue inflammation, and the body’s defense mechanisms [Data Sciences International 2001]. rhBMP-2/ACS-induced bone exhibited radiographic or histologic evidence of seroma formation in three animals. Seroma formation was not observed in the controls. The radiographic bone voids gradually became more radiopaque over the 8-week healing interval. Histologically, the bone voids appeared as empty vacuoles without a definable matrix. New bone formation was observed from the periphery of the vacuoles and lining the dental implants. These observations are consistent with previous observations in this model system [Sigurdsson et al. 1997; Tatakis et al. 2002] and following implantation of rhBMP-2/ACS into saddle-type intrabony defects in dogs [Hunt et al. 2001; Jovanovic et al. 2003].
The results of this study suggest that rhBMP-2/ACS significantly enhances the effect of GBR at turned and surface-etched dental implants. The dental implant surface technology does not appear to substantially influence bone formation.

Acknowledgements: The authors thank Melanie Manning, DVM, and Tim Dombrowski of W.L. Gore & Associates, Inc., for skilled and compassionate veterinary and technical animal care in the course of this study. The histological processing of the dental implant specimens was skillfully carried out by Hari S. Prasad, BS, MDT, University of Minnesota School of Dentistry. Implant Innovations, Inc. donated the dental implants custom-made for the supra-alveolar peri-implant defect model.

Conclusion

The results of this study suggest that rhBMP-2/ACS significantly enhances the effect of GBR at turned and surface-etched dental implants. The dental implant surface technology does not appear to substantially influence bone formation.

Zusammenfassung

rhBMP-2 steigert die gesteuerte Knochenergeneration signifikant.


Seroma formation has been observed following rhBMP-2-induced bone formation including a variety of carrier technologies and will gradually resolve to fill with bone to even serve for osseointegration and functional loading of dental implants (Sigurdsson et al. 2001; Jovanovic et al. 2003). Since seroma formation has also been observed following conventional surgical procedures without rhBMP-2, seromas may not be a unique effect of rhBMP-2 but rather an effect of biologic processes amplified by rhBMP-2.

Previous studies have indicated that the use of occlusive ePTFE devices for GBR in conjunction with rhBMP-2 technologies may accelerate new bone formation (Zellin & Linde 1997; Cochran et al. 1999). This study could not determine whether the macro-porous ePTFE device would have a similar effect. However, blood vessels penetrating the macro-porous device probably offered necessary neovascularity and cellular resources for rhBMP-2 function allowing rapid bone formation approximating the contours of the macro-porous device within the 8-week healing interval.

Table 3. Bone density and osseointegration (%) at turned and surface-etched dental implants in newly formed bone

<table>
<thead>
<tr>
<th></th>
<th>rhBMP-2/ACS</th>
<th>Buffer/ACS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bone density</td>
<td>BIC</td>
</tr>
<tr>
<td>Turned</td>
<td>26.5 ± 5.1</td>
<td>6.4 ± 1.5</td>
</tr>
<tr>
<td>Surface-etched</td>
<td>19.0 ± 4.2</td>
<td>9.6 ± 7.5</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0091</td>
<td>0.399</td>
</tr>
</tbody>
</table>

BIC = bone–implant contact.

Table 4. Bone density and osseointegration (%) at turned and surface-etched dental implants in resident bone

<table>
<thead>
<tr>
<th></th>
<th>rhBMP-2/ACS</th>
<th>Buffer/ACS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bone density</td>
<td>BIC</td>
</tr>
<tr>
<td>Turned</td>
<td>70.1 ± 4.0</td>
<td>43.3 ± 25.7</td>
</tr>
<tr>
<td>Surface-etched</td>
<td>67.5 ± 3.1</td>
<td>56.7 ± 12.5</td>
</tr>
<tr>
<td>P-value</td>
<td>0.118</td>
<td>0.191</td>
</tr>
</tbody>
</table>

BIC = bone–implant contact.
decken, spannte man eine formstabile, macroporöse ePTFE-Membran darüber. Zur Erreichung eines primären Wundverschlusses dehnte man die Gingiva lappen. 8 Wochen nach der Chirurgie wurden diese eingeschlägelt, um eine histologische und histometrische Analyse durchführen zu können.

**Resultate:** Bei Defekten, die einen rhBMP-2/ACS enthielten, verzeichnete man eine deutliche Steigerung der Knochenneubildung verglichen mit den Kontrollen. Der vertikale Knochengewinn lag in Tieren eingeschläfert, um eine histologische und histometrische Analyse durchführen zu können.

**Zusammenfassung:** Eine Kombination von rhBMP-2/ACS steigert die GBR sowohl bei glatten als auch bei säuregeätzten Oberflächen signifikant. Die Oberflächenbeschaffenheit scheint die Knochenneubildung nicht wesentlich zu beeinflussen.

**Resumen**

**Antecedentes:** Estudios previos han mostrado un potencial limitado para aumentar oseo tras reparación de defectos defectos de superficie gravada. La implantación quirúrgica de proteína-2 morfogénica osea recombinante humana (rhBMP-2) en una espontánea de colágeno absorbible como vehículos (ACS) realiza la regeneración ósea en dichos defectos, de todos modos no se obtienen rutinariamente cantidades suficientes de hueso para odontología de implantes. El objetivo de este estudio fue evaluar el potencial de rhBMP-2/ACS para realzar GBR usando un dispositivo macroporoso de polietilafluorooctileno expandido (e-PTFE) suministrado de espacio.

**Metodos:** Se crearon quirúrgicamente defectos periimplantarios bilaterales, de tamaño crítico, supraalveolares en cuatro perros mongrel Hound Labrador. Se colocaron dos implantes dentales de titanio de 10 mm pulidos y uno gravado con ácido en 5 mm del centro del hueso alveolar inducido. Se asignaron aleatoriamente rhBMP-2/ACS (rhBMP-2 a 0.2 mg/mL) o buffer/ACS a los cuadrantes izquierdos y derechos de la mandíbula en animales consecutivos. El dispositivo suministrador de espacio de ePTFE macro poroso se colocó para cubrir el rhBMP-2/ACS y las construcciones de control y los implantes dentales. Se elevaron colgajos gingivales para el cierre primario de la herida. Los animales se sacrificaron 8 semanas tras la cirugía para análisis histológico e histométrico.

**Resultados:** La formación de hueso fue significativamente realizada en los defectos que recibieron rhBMP-2/ACS en comparación con los controles. La ganancia vertical de hueso promedió (± SD) 4.7 ± 0.3 y 4.8 ± 0.1 mm, y el área de nuevo hueso 10.3 ± 2.0 y 8.0 ± 2.5 mm² en las superficies de los implantes dentales pulidos y las gravadas con ácido respectivamente. Los valores correspondientes para los controles fueron 1.8 ± 2.0 y 1.3 ± 1.3 mm, y 1.8 ± 1.3 ± 1.2 ± 0.6 mm². El contacto hueso-implante en el hueso inducido por rhBMP-2 promedió 6.4 ± 1.4 y 9.6 ± 7.5% para los implantes pulidos y con superficie gravada respectivamente (p = 0.390). Los valores correspondientes para los controles fueron 14.6 ± 19.4 y 23.7 ± 9.7% (p = 0.473). El contacto hueso implant en el hueso residente varió entre 43% y 58% sin diferencias significativas entre las superficies de los implantes dentales.

**Conclusiones:** El rhBMP-2/ACS realiza significativamente la GBR en implantes dentales de superficie pulida y gravada. Las superficies de los implantes dentales no parecen tener una influencia sustancial en la formación de hueso.

**References**


